

CLAIMS

1. A method of identifying in a eukaryotic target organism a nucleic acid sequence encoding a product that is involved in or is suspected of being involved in cell growth regulation
5 in said target organism, the method comprising the steps of:
 - (a) providing a wild type plant or a plant that is genetically modified to have tissue exhibiting, relative to the tissue of its non-genetically modified parent plant, accelerated growth,
10
 - (b) subjecting a multiplicity of the wild type plant or the genetically modified plant, or parts thereof to a mutagenisation treatment,
 - (c) selecting from the thus treated plants or parts thereof mutant plants having, relative to
15 the wild type plant or the genetically modified plant of step (a), a phenotype characterised by an altered morphological structure or an altered colour,
 - (d) identifying in said selected mutant plants nucleic acid sequence(s) having a nucleic acid sequence which is different from the corresponding sequence(s) in the non-mutagenised
20 wild type plant or the genetically modified plant, and, using said nucleic acid sequence(s),
 - (e) identifying in the eukaryotic target organism a target nucleic acid sequence comprising a sequence encoding a product that is involved in cell growth regulation.
25
2. A method according to claim 1, wherein the wild type plant or the parent plant for the genetically modified plant of step (a) is selected from a group consisting of *Lotus japonicus*, *Medicago truncatula*, *Oryza sativa*, *Antirrhinum majus* and *Arabidopsis thaliana*.
30
3. A method according to claim 2, wherein the wild type plant or the parent plant is *Arabidopsis thaliana*.
35
4. A method according to any of claims 1-3, wherein the accelerated growth of tissue of the genetically modified plant is due to overexpression of a gene selected from the group consisting of a gene coding for a cyclin, a gene coding for a transcription factor including E1A, E2F, myc, and any other gene positively affecting the cell cycle regulatory system.

5. A method according to claim 4, wherein the gene coding for a cyclin is selected from the group consisting of a gene encoding a cyclin of B-type and a gene encoding a cyclin of D type.
- 5 6. A method according to any of claims 1-5, wherein the genetically modified plant is obtained by introducing into a cell of the parent plant a gene construct comprising a promoter and, operably linked thereto, a nucleotide sequence encoding a gene product that is involved in acceleration of growth in a tissue of the thus modified plant.
- 10 7. A method according to claim 6, wherein said gene product activates the cell cycle regulatory system of the plant.
8. A method according to claim 7, wherein the gene product is encoded by a gene selected from the group consisting of a gene coding for a cyclin, a gene coding for a transcription factor including E2F and myc, and any other gene positively affecting the cell cycle regulatory system.
- 15 9. A method according to claim 8, wherein the gene coding for cyclin is selected from the group consisting of the cyc1At gene (encoding a mitotic cyclin of B-type), the AtcycD2 gene (encoding a G1 cyclin of D-type) and the AtcycD1 gene (also encoding a G1 cyclin).
- 20 10. A method according to claim 6, wherein the promoter is a plant gene promoter.
11. A method according to claim 10, wherein the promoter is selected from the group consisting of an inducible promoter and a constitutive promoter.
- 25 12. A method according to claim 10, wherein the plant gene promoter is selected from the group consisting of an Atcdc2a promoter (prAtcdc2a), a 3SS promoter and an Atcdc2b promoter.
- 30 13. A method according to claim 6 wherein the gene construct comprises a poly-adenylation site.
14. A method according to claim 13, wherein the poly-adenylation site is derived from the Nopaline synthetase gene of *Agrobacterium tumefaciens*, an octopine synthetase gene or 35S polyadenylation sequences.
15. A method according to any of claims 6-14, wherein the gene construct is introduced by means of *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*.

16. A method according to any of the preceding claims, wherein the mutagenisation treatment of step (b) is performed by a method selected from the group consisting of EMS mutagenesis, T-DNA-mutagenesis and mutagenesis by using a transposable element.

5

17. A method according to any of the preceding claims, wherein the identification in step (d) of nucleic acid sequence(s) having a sequence which is different from the corresponding sequence(s) in the non-mutagenised transgenic plant is performed using a method selected from the group consisting of an Amplified Fragment Length Polymorphism (AFLP)

10 method, a Single Sequence Length Polymorphism (SSLP), a differential display method, a restriction fragment length polymorphism (RFLP) method, a Single Strand Conformation Polymorphism (SSCP) method, allele specific amplification, restriction PCR, PCR, sequencing and a Single Nucleotide Polymorphism (SNP) method.

15 18. A method according to claim 17, wherein the identification method is an SSLP method.

19. A method according to any of the preceding claims, wherein the nucleic acid sequence identified in step (d) and/or the product encoded by the sequence is functionally associated with the phenotype of the selected mutant plants of step (c).

20. A method according to any of the preceding claims, wherein, in step (e), the target nucleic acid sequence is identified by a homology search in a genome database for the target organism or by molecular probing.

25

21. A method according to claims 20, wherein the molecular probing is carried out using a method selected from the group consisting of PCR, northern blotting, Southern blotting, arraying and direct sequencing.

30 22. A method according to any of the preceding claims comprising the further step of isolating the target nucleic acid sequence identified in step (e).

23. A method according to any of the preceding claims, wherein the product of the target nucleic acid sequence is functionally active in a signal transduction cascade leading to suppression of cell growth in the target organism.

35 24. A method according to any of the preceding claims, wherein the product of the target nucleic acid sequence is a suppressor of cell growth in the target organism.

25. A method according to any of the preceding claims, wherein a putative functional association between the plant nucleic acid sequence identified in step (d) and the target nucleic acid sequence is determined by homology analysis between said plant nucleic sequence and said target nucleic sequence.

5

26. A method according to any of the preceding claims, wherein a putative functional association between the plant nucleic acid sequence identified in step (d) and the target nucleic acid sequence is determined by analysing the effect of expressing the target nucleic acid sequence in an *in vitro* model for assaying cell growth regulation activity.

10

27. A method according to any of the preceding claims, wherein a putative functional association between the plant nucleic acid sequence identified in step (d) and the target nucleic acid sequence is determined by analysing the effect of expressing the target nucleic acid sequence in an *in vivo* model for assaying cell growth regulation activity.

15

28. A method according to any of the preceding claims, wherein the eukaryotic target organism is a cell selected from the group consisting of a microbial cell, a plant cell and a mammalian cell.

20 29. A method according to claim 28, wherein the microbial cell is a yeast cell.

30. A method according to claim 28, wherein the mammalian cell is a cell of a mammal selected from the group consisting of insects, birds, mice, rats, guinea pigs, cats, dogs, apes, primates including humans.

25

31. A method according to any of claims 1-30, wherein the plant that is provided in step (a) is a wild type plant.

32. A method of determining the tumour suppressor activity, if any, of a gene product encoded by a eukaryotic cell gene and suspected of having tumour suppressor activity, the method comprising the steps of:

(a) providing a wild type plant or a plant that is genetically modified to have tissue exhibiting, relative to the tissue of its non-genetically modified parent plant, accelerated growth,

35

(b) subjecting a multiplicity of the wild type plant or the genetically modified plant or parts thereof to a mutagenisation treatment,

(c) selecting from the thus treated plants or parts thereof a mutant plant having, relative

to the wild type plant or the genetically modified plant of step (a), a phenotype characterised by an altered morphological structure or an altered colour,

-
-
-
-
- (d) identifying in said selected mutant plant a nucleic acid sequence having a sequence which is different from the corresponding sequence in the non-mutagenised wild type plant or genetically modified plant, and, using said different nucleic acid, identifying in the eukaryotic cell a homologue or analogue gene putatively involved in cell cycle regulation,
- 10 (e) transforming the coding sequence of said homologue or analogue gene into a mutant plant of step (c) under conditions permitting the sequence to be expressed, and
-
- (f) determining whether or not the thus transformed mutant plant reverts to its wild type phenotype, such reversion being indicative of tumour suppressor activity of the homologue
- 15 or analogue gene product.